

AMENDMENTS**Amendments to the Claims**

1-3. (Canceled)

4. (Previously presented) A botulinum toxin serotype A (BoNT/A) substrate, comprising:

(a) a donor fluorophore;

(b) an acceptor having an absorbance spectrum overlapping the emission spectrum of said donor fluorophore; and

(c) a BoNT/A recognition sequence comprising a cleavage site;

wherein said cleavage site intervenes between said donor fluorophore and said acceptor;

wherein at least 14 amino acids separate said donor fluorophore from said acceptor; and

wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor.

5. (Original) The substrate of claim 4, comprising at least six consecutive residues of SNAP-25, said six consecutive residues comprising Gln-Arg, or a peptidomimetic thereof.

6. (Previously presented) The substrate of claim 5, comprising at least six consecutive residues of a human SNAP-25, said six consecutive residues comprising Gln197-Arg198, or a peptidomimetic thereof.

7. (Original) The substrate of claim 6, comprising the amino acid sequence Glu-Ala-Asn-Gln-Arg-Ala-Thr-Lys (SEQ ID NO: 1), or a peptidomimetic thereof.

8. (Original) The substrate of claim 6, comprising residues 187 to 203 of human SNAP-25 (SEQ ID NO: 2), or a peptidomimetic thereof.

9-44. (Canceled)

45. (Previously presented) The substrate of claim 4, wherein said substrate can be cleaved with an activity of at least 1 nanomole/minute/milligram toxin.

46. (Previously presented) The substrate of claim 4, wherein said substrate can be cleaved with an activity of at least 20 nanomole/minute/milligram toxin.

47. (Previously presented) The substrate of claim 4, wherein said substrate can be cleaved with an activity of at least 50 nanomole/minute/milligram toxin.

48. (Previously presented) The substrate of claim 4, wherein said substrate can be cleaved with an activity of at least 100 nanomole/minute/milligram toxin.
49. (Previously presented) The substrate of claim 4, wherein said substrate can be cleaved with an activity of at least 150 nanomole/minute/milligram toxin.
50. (Previously presented) The substrate of claim 4, wherein said acceptor is an acceptor fluorophore.
51. (Original) The substrate of claim 50, wherein said acceptor fluorophore has a fluorescent lifetime of at least 1 microsecond.
52. (Previously presented) The substrate of claim 4, wherein said acceptor is non-fluorescent.
53. (Previously presented) The substrate of claim 4, wherein said donor fluorophore is fluorescein.
54. (Canceled)
55. (Previously presented) The substrate of claim 4, wherein said donor fluorophore is 4-(4-dimethylaminophenylazo)benzoic acid (DABCYL) DABCYL.
56. (Canceled)
57. (Previously presented) The substrate of claim 4 or claim 53, wherein said acceptor is tetramethylrhodamine.
58. (Previously presented) The substrate of claim 4 or claim 55, wherein said acceptor is 5-[(2-aminoethyl)amino]-naphthalene-1-sulfonic acid (EDANS) EDANS.
59. (Previously presented) The substrate of claim 4 or claim 53, wherein said acceptor is a non-fluorescent acceptor.
60. (Previously presented) The substrate of claim 4, which is a peptide or peptidomimetic having at most 100 residues.
61. (Previously presented) The substrate of claim 4, which is a peptide or peptidomimetic having at most 50 residues.
62. (Previously presented) The substrate of claim 4, which is a peptide or peptidomimetic having at most 40 residues.
63. (Previously presented) The substrate of claim 4, which is a peptide or peptidomimetic having at most 20 residues.
64. (Previously presented) The substrate of claim 50, wherein said donor fluorophore and said acceptor fluorophore are separated by at most fifteen residues.

65-95. (Canceled)

96. (Previously presented) The substrate of claim 4, wherein said substrate has a length of 19 amino acids.

97. (Previously presented) The substrate of claim 4, wherein said substrate has a length of 20 amino acids.

98. (Previously presented) The substrate of claim 4, wherein said substrate has a length of 21 amino acids.

99. (Previously presented) The substrate of claim 4, wherein said substrate has a length of 22 amino acids.

100. (Previously presented) The substrate of claim 4, wherein said substrate has a length of 69 amino acids.

101. (Previously presented) The substrate of claim 4, wherein said substrate has a length of 72 amino acids.

102. (Currently amended) A botulinum toxin serotype A (BoNT/A) substrate, comprising:

(a) a donor fluorophore;

(b) an acceptor having an absorbance spectrum overlapping the emission spectrum of said donor fluorophore; and

(c) a BoNT/A recognition sequence comprising a cleavage site, said BoNT/A recognition sequence comprising the amino acids 191 to 202 of SEQ ID NO: 2 ~~sequence Arg-Ile-Asp-Glu-Ala-Asn-Gln-Arg-Ala-Thr-Lys-Met (SEQ ID NO: 2)~~, or a peptidomimetic thereof;

wherein said cleavage site intervenes between said donor fluorophore and said acceptor;

wherein said acceptor is not positioned within amino acids 191 to 202 of SEQ ID NO: 2 ~~SEQ ID NO: 2~~; and

wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor.

103. (Previously presented) The substrate of claim 102, comprising the amino acid sequence Ser-Asn-Lys-Thr-Arg-Ile-Asp-Glu-Ala-Asn-Gln-Arg-Ala-Thr-Lys-Met (SEQ ID NO: 29), or a peptidomimetic thereof.

104. (Previously presented) The substrate of claim 102, comprising the amino acid sequence Ser-Asn-Lys-Thr-Arg-Ile-Asp-Glu-Ala-Asn-Gln-Arg-Ala-Thr-Lys-Met-Leu (SEQ ID NO: 30), or a peptidomimetic thereof.

105. (Previously presented) The substrate of any of claims 102, 103 or 104, wherein said substrate can be cleaved with an activity of at least 1 nanomole/minute/milligram toxin.
106. (Previously presented) The substrate of any of claims 102, 103 or 104, wherein said substrate can be cleaved with an activity of at least 20 nanomole/minute/milligram toxin.
107. (Previously presented) The substrate of any of claims 102, 103 or 104, wherein said substrate can be cleaved with an activity of at least 50 nanomole/minute/milligram toxin.
108. (Previously presented) The substrate of any of claims 102, 103 or 104, wherein said substrate can be cleaved with an activity of at least 100 nanomole/minute/milligram toxin.
109. (Previously presented) The substrate of any of claims 102, 103 or 104, wherein said substrate can be cleaved with an activity of at least 150 nanomole/minute/milligram toxin.
110. (Previously presented) The substrate of claim 102, wherein said acceptor is an acceptor fluorophore.
111. (Previously presented) The substrate of claim 110, wherein said acceptor fluorophore has a fluorescent lifetime of at least 1 microsecond.
112. (Previously presented) The substrate of claim 102, wherein said acceptor is a non-fluorescent acceptor.
113. (Previously presented) The substrate of claim 102, wherein said donor fluorophore is fluorescein.
114. (Previously presented) The substrate of claim 102, wherein said donor fluorophore is DABCYL.
115. (Previously presented) The substrate of claim 102 or 113, wherein said acceptor is tetramethylrhodamine.
116. (Previously presented) The substrate of claim 102 or 114, wherein said acceptor is EDANS.
117. (Previously presented) The substrate of claim 113, wherein said acceptor is a non-fluorescent acceptor.
118. (Previously presented) The substrate of claim 102, which is a peptide or peptidomimetic having at most 100 residues.
119. (Previously presented) The substrate of claim 102, which is a peptide or peptidomimetic having at most 50 residues.
120. (Previously presented) The substrate of claim 102, which is a peptide or peptidomimetic having at most 40 residues.

121. (Previously presented) The substrate of claim 102, which is a peptide or peptidomimetic having at most 20 residues.
122. (Previously presented) The substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most fifteen residues.
- 123-125. (Canceled)
126. (Previously presented) A botulinum toxin serotype A (BoNT/A) substrate, comprising:
- (a) a donor fluorophore;
 - (b) an acceptor having an absorbance spectrum overlapping the emission spectrum of said donor fluorophore; and
 - (c) a BoNT/A recognition sequence comprising a cleavage site, wherein said cleavage site intervenes between said donor fluorophore and said acceptor;
- wherein said donor fluorophore or said acceptor is genetically encoded; and
- wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor.
127. (Previously presented) The substrate of claim 126, wherein said donor fluorophore is genetically encoded.
128. (Previously presented) The substrate of claim 126, wherein said acceptor is genetically encoded.
129. (Previously presented) The substrate of claim 126, wherein said donor fluorophore and said acceptor are genetically encoded.
130. (Previously presented) The substrate of claim 126, comprising at least six consecutive residues of SNAP-25, said six consecutive residues comprising Gln-Arg.
131. (Previously presented) The substrate of claim 130, comprising at least six consecutive residues of a human SNAP-25, said six consecutive residues comprising Gln197-Arg198.
132. (Previously presented) The substrate of claim 131, comprising the amino acid sequence Glu-Ala-Asn-Gln-Arg-Ala-Thr-Lys (SEQ ID NO: 1).
133. (Previously presented) The substrate of claim 131, comprising residues 187 to 203 of human SNAP-25 (SEQ ID NO: 2).
134. (Previously presented) The substrate of claim 126, wherein said substrate can be cleaved with an activity of at least 1 nanomole/minute/milligram toxin.

135. (Previously presented) The substrate of claim 126, wherein said substrate can be cleaved with an activity of at least 20 nanomole/minute/milligram toxin.
136. (Previously presented) The substrate of claim 126, wherein said substrate can be cleaved with an activity of at least 50 nanomole/minute/milligram toxin.
137. (Previously presented) The substrate of claim 126, wherein said substrate can be cleaved with an activity of at least 100 nanomole/minute/milligram toxin.
138. (Previously presented) The substrate of claim 126, wherein said substrate can be cleaved with an activity of at least 150 nanomole/minute/milligram toxin.
139. (Previously presented) The substrate of claim 126, wherein said acceptor is an acceptor fluorophore.
140. (Previously presented) The substrate of claim 139, wherein said acceptor fluorophore has a fluorescent lifetime of at least 1 microsecond.
141. (Previously presented) The substrate of claim 126, which is a peptide having at most 400 residues.
142. (Previously presented) The substrate of claim 126, which is a peptide having at most 500 residues.
143. (Previously presented) The substrate of claim 126, which is a peptide having at most 600 residues.
144. (Previously presented) The substrate of claim 126 which is a peptide having at most 700 residues.
145. (Previously presented) The substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most fifteen residues.
146. (Previously presented) The substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most ten residues.
147. (Previously presented) The substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most eight residues.
148. (Previously presented) The substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most six residues.
149. (Previously presented) The substrate of claim 4, wherein said donor fluorophore and said acceptor are separated by at most twenty residues.
150. (Previously presented) The substrate of claim 4, wherein said donor fluorophore and said acceptor are separated by at most twenty-five residues.

151. (Previously presented) The substrate of claim 4, wherein said donor fluorophore and said acceptor are separated by at most thirty residues.
152. (Previously presented) The substrate of claim 4, wherein said donor fluorophore and said acceptor are separated by at most thirty-five residues.
153. (Previously presented) The substrate of claim 4, wherein said donor fluorophore and said acceptor are separated by at most forty residues.
154. (Previously presented) The substrate of claim 4, selected from the group consisting of SEQ ID NO: 85, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94 and SEQ ID NO: 95.
155. (Previously presented) The substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most twenty residues.
156. (Previously presented) The substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most twenty-five residues.
157. (Previously presented) The substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most thirty residues.
158. (Previously presented) The substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most thirty-five residues.
159. (Previously presented) The substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most forty residues.
160. (Previously presented) The substrate of claim 126, which is a peptide or peptidomimetic having at least 300 residues.
161. (Previously presented) The substrate of claim 126, which is a peptide or peptidomimetic having at least 400 residues.
162. (Previously presented) The substrate of claim 126, which is a peptide or peptidomimetic having at least 500 residues.
163. (Previously presented) The substrate of claim 126, which is a peptide or peptidomimetic having at least 600 residues.
164. (Previously presented) The substrate of claim 126, which is a peptide or peptidomimetic having at least 700 residues.
165. (Previously presented) The substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most twenty residues.
166. (Previously presented) The substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most twenty-five residues.

167. (Previously presented) The substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most thirty residues.
168. (Currently amended) The substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most forty ~~thirty-five~~ residues.
169. (Currently amended) The substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most fifty ~~forty~~ residues.
170. (Previously presented) The substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 50 residues.
171. (Previously presented) The substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 75 residues.
172. (Previously presented) The substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 100 residues.
173. (Previously presented) The substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 125 residues.
174. (Previously presented) The substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 150 residues.
175. (Previously presented) The substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 200 residues.
176. (New) The substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most ten residues.
177. (New) The substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most eight residues.
177. (New) The substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most six residues.